Pestic. Sci. 1996, **46**, 191–197

The Effects of the Duration of Exposure on the Toxicity of Diflubenzuron, Hexaflumuron and Teflubenzuron to Various Stages of II Instar *Schistocerca gregaria*

George D. A. Coppen* & Paul C. Jepson‡

Department of Biology, University of Southampton, Biomedical Sciences Building, Bassett Crescent East, Southampton SO16 7PX, UK

(Received 23 March 1995; revised version received 21 August 1995; accepted 18 September 1995)

Abstract: Second-instar (II) nymphs of the Desert Locust, *Schistocerca gregaria* (Forsk.) were exposed to three benzoylphenyl ureas (BPUs), diflubenzuron, hexaflumuron and teflubenzuron. Nymphs were treated with precise doses by allowing them to ingest treated barley leaves at varying stages of the II instar. They were exposed to the same total quantity of active ingredient over one (Days 1, 2, 3 or 4), two (Days 1–2, 2–3 or 3–4) or four days (Days 1–4) of the four-day inter-moult period. The total amounts applied were 60 μg per nymph of diflubenzuron, 30 μg per nymph of hexaflumuron or 0·25 μg per nymph of teflubenzuron. The nymphs were then monitored for two moults after treatment until they reached the fourth (IV) instar, to observe both the acute and chronic effects of treatment. The timing of the exposure during the inter-moult period and the duration of exposure were both found to result in significantly different acute responses for each BPU. Treatment over one or two days showed that the closer to the moult II instars were treated, the greater the mortality. This indicated that locust pharate cuticle is predominantly synthesised late in the instar. Treatment over four days resulted in higher mortality than exposure to the same quantity of active ingredient over one or two days, suggesting that BPUs are highly toxic to locust nymphs but non-cumulative within their bodies. The timing of death was also significantly affected by both the timing and duration of treatment. A significant proportion of the mortality occurred after the first moult following treatment when nymphs were dosed on Day 1 and Day 2 with hexaflumuron and diflubenzuron respectively. Mortality following all other treatments occurred during the first moult after treatment. The duration of the II and third (III) instars were significantly prolonged in many cases following treatment with BPUs. The implications of increased mortality following prolonged exposure to BPUs and extended development periods are discussed in relation to the use of BPUs as barrier-sprayed insecticides for the control of mobile locust nymph populations.

Key words: diflubenzuron, hexaflumaron, teflubenzuron, insect growth regulator, *Schistocerca gregaria*, desert locust.

1 INTRODUCTION

The benzoylphenyl ureas insecticides (BPUs) diflubenzuron, hexaflumuron and teflubenzuron are all toxic to *Schistocerca gregaria* (Forsk.) nymphs by the inhibition of chitin deposition.[1,2] They persist in the field for between two weeks (teflubenzuron) and two months (diflubenzuron)[3,4] and have become candidates for the control of locusts and grasshoppers in sahelian grassland.[5,6] BPUs are not cumulative within the locust's body and, once eliminated, chitin deposition resumes normally; diflubenzuron has a half-life of about 24 h in adult in *S. gregaria*.[7] Exposure to BPUs at *different*

* To whom correspondence should be addressed at: Cyanamid International, Agricultural Products Division, Chaussée de Tirlemont 105, B-5030 Gembloux, Belgium.
‡ Present address: Department of Entomology, Oregon State University, Cordley Hall, Corvallis 97331, OR, USA.

Pestic. Sci. 0031-613X/96/$09.00

TABLE 1
Percentage Mortality after Ingesting a Single BPU Dose on One Inter-Moult Day of the II Instar[a]

BPU	*Inter-moult application (day)*	*Dose applied (g AI litre^{-1})*	*Observed mortality (%)*
Diflubenzuron	1	40	7
	2	40	33
	3	40	60
	4	40	100
Hexaflumuron	1	30	26
	2	30	95
	3	30	100
	4	30	98
Teflubenzuron	1	1	0
	2	1	30
	3	1	73
	4	1	100

[a] Predicted dose response mortality (%) (±95% CI) from a single dose on Day 2 of diflubenzuron 38(29–49); hexaflumuron 55(49–61); teflubenzuron 27(13–47).[10]

stages[7,8] and for different durations[9] during the inter-moult period is liable to give rise to different mortality rates. Diflubenzuron, hexaflumuron and teflubenzuron have been shown to be toxic to II instar *S. gregaria* nymphs following a single dose by ingestion at a specific age.[10,11] Changes in either timing or duration are, however, liable to affect toxicity, and these insecticides were evaluated further in the present study to determine their toxicities when the duration and timing of exposure were varied.[7,8,12,13] The aim of this study was to help delimit any periods of susceptibility or 'windows of opportunity' during the inter-moult stages. Such data will be useful in determining model parameters that predict optimum spray application rates for the use of BPUs against locusts and grasshoppers in the field and also aiding interpretation of field experiments where such phenomena could lead to variable efficacy.

2 MATERIALS AND METHODS

2.1 Locust culture

A laboratory culture of *S. gregaria* was maintained in a constant environment of 25–38°C with 40–60% RH and 12:12 h light:dark and provided with excess spring barley and dry wheat bran.[10] The selected culture conditions gave repeatable and reliable locust development rates. It was therefore possible to predict moulting times and the days when nymphs would feed and take in doses of insecticide presented on foliage. For the II instar there were four days when nymphs would feed.

2.2 Insecticides

Diflubenzuron ('Dimilin' 450 g litre^{-1} ODC), hexaflumuron ('Consult' 250 g litre^{-1} ODC) and teflubenzuron ('Nomolt' 10 g litre^{-1} ULV) were supplied by Solvay Duphar, The Netherlands; DowElanco, Europe and Shell Research Ltd, UK respectively. The insecticide formulation techniques used were the same as those described previously.[11]

2.3 Experimental protocol

It was hypothesised that a BPU dose applied at different times and/or for different durations during the inter-moult period would result in different mortality rates. In order to test this, a predetermined dose estimated to be the LD_{30} for each BPU was orally applied by one of three regimes, making eight treatments plus controls, using the dosing techniques described previously.[11] For the one-day exposure, nymphs were supplied with the entire dose (100% of LD_{30}) on a short piece (1·5–2 cm) of young spring barley for consumption within the designated inter-moult day. A two-day exposure duration was supplied in two equal and separate daily doses consecutively (50% of the LD_{30} on each day). The four-day exposure was administered in four separate doses on successive days (25% of the LD_{30} on each day). All treated material was consumed before the test insects were returned to the specified cage for that dose regime. When exposed to treated vegetation, nymphs were regularly inspected so that individuals that had completed the dose could be returned to the cage and feed at will. Those individuals that did not consume all treated material within the designated time were discarded. A minimum of 50 test insects were used for each treatment, with a similar number for controls which were dosed with blank formulation or provided with untreated leaves. The insecticide doses were applied within several stock formulations: 40 and 60 g AI litre^{-1} of diflubenzuron, 10 and 30 g AI litre^{-1} of hexaflumuron and 0·25 and 1·0 g AI litre^{-1} of teflubenzuron. The doses were 60 μg per nymph of diflubenzuron, 30 μg per nymph of hexaflumuron or 0·25 μg per nymph of teflubenzuron. It had been demonstrated that these were equivalent to LD_{40}, LD_{55} and LD_{27} respectively.[11] The monitoring techniques were the same as those described previously.[11]

2.4 Analysis

The observed effects were plotted against the effects obtained in the single dosing regime.[11] Deviations from the expected effect range should have been detectable if observed values fell outside the range of confidence intervals for single-dose toxicological assays. This should (i) indicate whether the mortality following an alternative dose regime was significantly different

TABLE 2
χ^2 Analysis of Mortality Classes from a Single Dose on One Inter-moult Day

BPU	Regime code	Observed frequencies of mortality classes: In moult	Post-moult	χ^2 value (1df)[a]
Diflubenzuron	Day 1	1	3	1[ns]
	Day 2	0	8	7·5**
	Day 3	8	27	18***
	Day 4	45	0	44***
	Totals:	54	38	2·4[ns]
Hexaflumuron	Day 1	0	10	9**
	Day 2	39	15	23***
	Day 3	36	2	33***
	Day 4	59	0	58***
	Totals:	134	27	70***
Teflubenzuron	Day 1	0	0	1[ns]
	Day 2	34	1	32***
	Day 3	57	1	55***
	Day 4	40	0	39***
	Totals:	131	2	122***

[a] Significance of difference between in moult and post-moult: *** $P < 0.001$; ** $P < 0.01$; [ns] $P > 0.05$.

($P < 0.05$) from the 'single shot' dose–response estimate and (ii) reveal any trends in mortality as a function of physiological age at dosing. The frequencies of observed mortality in 'in moult' and 'post-moult' classes were compared against expected values by chi-square test. Comparisons were also made between mean inter-moult periods (days ±95% CI) for both II and III instars to detect any significant sub-lethal effects.

3 RESULTS

3.1 Effects following a single dose on one inter-moult day

3.1.1 Acute effects

All three compounds showed a progressive increase in mortality the later a dose was applied to II instar nymphs before they moulted to the III instar (Table 1). There was a constant increase in mortality between consecutive treatment days for diflubenzuron- and teflubenzuron-treated nymphs. In both cases mortality resulting from the Day 2 dose fell within the original dose–response confidence limits. This indicates that the test insects exhibited similar patterns of tolerance to those found previously.[11] Hexaflumuron-treated nymphs showed a disproportionate increase in mortality with a low Day 1 dosing mortality, but high Day 2, Day 3 and Day 4 dosing mortalities.

3.1.2 Mortality classes

There were significant biases in the timing of death relative to moulting in all the treatments except Day 1 diflubenzuron and teflubenzuron (Table 2). Days 2 & 3 diflubenzuron and Day 1 hexaflumuron gave significantly more mortality in the post-moult class ($P < 0.05$, 0·01 & 0·05 respectively). All other treatment timings resulted in significantly more mortality in moult ($P < 0.01$ in all cases).

3.1.3 Developmental periods

The inter-moult periods of II and III instars were in some cases extended (Table 3). Day 1 diflubenzuron, Days 2 & 3 hexaflumuron and Days 1, 2 and 4 teflubenzuron all resulted in II instar inter-moult periods that

TABLE 3
Mean Days (±95% CI) to Moult to III and IV Instar after Ingesting a Single BPU Dose on One Inter-Moult Day of the II Instar

BPU	Regime code	Mean days (±95% CI) to III instar[a]	Number of nymphs observed	Mean days (±95% CI) to IV instar[a]	Number of nymphs observed
Diflubenzuron	Control	5.4 (±0·1)	54	9·6 (±0·1)	54
	Day 1	5·8 (±0·1)*	59	11·1 (±0·4)*	59
	Day 2	5·0 (±0·0)*	58	9·4 (±0·2)[ns]	55
	Day 3	5·5 (±0·1)[ns]	57	12·3 (±0·8)*	28
	Day 4	5·5 (±0·2)[ns]	45	—	—
Hexaflumuron	Control	6·2 (±0·2)	59	10·7 (±0·2)	58
	Day 1	6·2 (±0·2)[ns]	59	11·2 (±0·4)[ns]	47
	Day 2	6·8 (±0·1)*	55	—	—
	Day 3	6·7 (±0·1)*	57	—	—
	Day 4	6·5 (±0·2)[ns]	60	—	—
Teflubenzuron	Control	5·1 (±0·1)	80	—	—
	Day 1	5·6 (±0·1)*	77	—	—
	Day 2	5·6 (±0·1)*	115	—	—
	Day 3	5·0 (±0·1)[ns]	41	—	—
	Day 4	5·8 (±0·2)*	40	—	—

[a] Significantly different from control at: *** $P < 0.001$; ** $P < 0.01$; [ns] $P > 0.05$.

TABLE 4
Percentage Mortality after Ingesting Two or Four BPU Doses on Consecutive Days of the II Instar. Predicted Dose Response Mortality as for Table 1

BPU	Inter-moult application (days)	Dose applied (g AI litre^{-1})	Observed mortality (%)
Diflubenzuron	1–2	60	5
	2–3	60	83
	3–4	60	94
	1–4	60	85
Hexaflumuron	1–2	10	15
	2–3	10	43
	3–4	10	99
	1–4	10	99
Teflubenzuron	1–2	0·25	20
	2–3	0·25	73
	3–4	0·25	100
	1–4	0·25	97

were significantly longer than those of the control ($P < 0·05$). Day 2 diflubenzuron-treated nymphs moulted to III instars significantly faster than the control nymphs ($P < 0·05$). The time taken by II instars to moult to the IV instar was also significantly longer than that of the control following treatment with diflubenzuron on Days 1 and 3 of the II instar. Dosing on Day 2 with diflubenzuron and on Day 1 with hexaflumuron did not significantly affect inter-moult periods ($P > 0·05$). Other exposure days either resulted in too few individuals to monitor to the IV instar or monitoring did not extend beyond the III instar.

TABLE 5
χ^2 Analysis of Mortality Classes after Two or Four BPU Doses on Consecutive Days of the II Instar

BPU	Regime code	Observed frequencies of mortality classes: In moult	Observed frequencies of mortality classes: Post-moult	χ^2 value (1df)[a]
Diflubenzuron	Days 1–2	3	0	2^{ns}
	Days 2–3	42	8	33***
	Days 3–4	17	0	16***
	Days 1–4	28	0	26***
	Totals:	90	8	67***
Hexaflumuron	Days 1–2	2	2	1^{ns}
	Days 2–3	9	0	8**
	Days 3–4	87	0	86***
	Days 1–4	87	2	84***
	Totals:	185	4	171***
Teflubenzuron	Days 1–2	8	8	1^{ns}
	Days 2–3	24	0	23***
	Days 3–4	30	0	29***
	Days 1–4	29	1	27***
	Totals:	91	9	66***

[a] Significance of difference between in moult and post-moult: *** $P < 0·001$; ** $P < 0·01$; ^{ns} $P > 0·05$.

3.2 Effects following two or four doses applied on consecutive inter-moult days

3.2.1 Acute effects

A progressive increase in mortality was observed the closer to the III instar moult that BPU doses were applied over Days 1–2, 2–3 and 3–4 during the II instar and exposure over Days 1–4 gave consistently high

TABLE 6
Mean Days (±95% CI) to Moult to III and IV Instar after Ingesting Two or Four BPU Doses on Consecutive Days of the II Instar[a]

BPU	Regime code	Mean days (±95% CI) to III instar	Number of nymphs observed	Mean days (±95% CI) to IV instar	Number of nymphs observed
Diflubenzuron	Control	5·0 (±0·1)	92	9·0 (±0·1)	58
	Days 1–2	5·1 (±0·1)^{ns}	59	9·6 (±0·2)*	56
	Days 2–3	5·0 (±0·0)^{ns}	60	11·1 (±0·2)*	9
	Days 3–4	5·7 (±0·4)*	18	—	—
	Days 1–4	5·6 (±0·2)*	33	—	—
Hexaflumuron	Control	5·4 (±0·1)	83	9·8 (±0·1)	58
	Days 1–2	6·5 (±0·3)*	26	11·8 (±0·4)*	20
	Days 2–3	7·0 (±0·1)*	20	—	—
	Days 3–4	6·2 (±0·2)*	27	—	—
	Days 1–4	6·2 (±0·2)*	90	—	—
Teflubenzuron	Control	5·3 (±0·1)	110	10·1 (±0·1)	27
	Days 1–2	5·5 (±0·3)^{ns}	71	11·9 (±0·2)*	34
	Days 2–3	6·3 (±0·2)*	33	12·1 (±0·6)*	8
	Days 3–4	5·2 (±0·2)^{ns}	29	—	—
	Days 1–4	6·6 (±0·3)*	30	—	—

[a] Significance values as Table 3.

mortality (Table 4). Mortality following exposure to diflubenzuron on Days 1–2 and hexaflumuron on Days 1–2 and 2–3 all fell below the LD_{50} value 95% confidence limits determined from the previous dose response study.[11] Mortality following dosing with teflubenzuron on Days 1–2 fell inside the dose–response confidence intervals whereas mortalities after dosing with diflubenzuron or teflubenzuron on Days 2–3 and 3–4 and all three BPUs on Days 3–4 and 1–4 were significantly greater than the confidence intervals for single-dose treatments.

3.2.2 Mortality classes

The mortality class in moult contained significantly more observations than the post-moult class following every application regime except for days 1–2 for all BPUs (Table 5).

3.2.3 Developmental periods

The inter-moult duration of some nymphs treated with two or four doses of a BPU was significantly different from that of the associated control during the II–III and III–IV moults (Table 6). There were no significant differences in the duration of the II instar period between nymphs treated with diflubenzuron on Days 1–2 and 2–3 and teflubenzuron on Days 1–2 and 3–4 and the controls. II instar nymphs moulted to III instar significantly more slowly when treated with diflubenzuron on Days 3–4 and 1–4; hexaflumuron with all applications and with teflubenzuron when dosed on Days 2–3 and 1–4. All survivors of these treatment regimes that were monitored until they moulted to IV instar took significantly longer to moult than the controls, with the exception of Days 2–3 diflubenzuron. These regimes were: diflubenzuron and teflubenzuron treated on Days 1–2 and 2–3 and hexaflumuron treated on Days 1–2.

4 DISCUSSION

When II instar nymphs were dosed over one or two days it was found that, the closer to the following moult an application was made, the greater the mortality. These results indicate that BPUs are highly toxic but non-cumulative and that chitin synthesis occurs late in the instar. They also suggest that diflubenzuron, hexaflumuron and teflubenzuron are quickly eliminated from the nymphs' bodies, an observation made in other insect species[14,15] as well as locusts.[16] The very high mortality obtained from the four-day exposure regime supports these hypotheses because, being applied on a daily basis throughout the instar, the insecticide level was maintained at a low level for a long period.

The highest mortality resulted from treatments made approaching the next moult. This indicated that chitin deposition may peak just before moulting, on Days 3 and 4 of the II instar, which is in agreement with Ker,[7] who found that most pharate cuticle of V instars was deposited during the last two days before moulting. The lower mortalities occurring in earlier-ingested applications suggest that BPUs did not persist within the locust bodies for sufficiently long to inhibit chitin synthesis at its peak, near the end of the inter-moult stage. The high mortality level found after a single hexaflumuron dose on Day 2 is an exception that may have resulted from formulation effects and evidence obtained by Coppen & Jepson[11] found that the formulation is an additional factor that can affect the toxicity of some BPUs.[8] Similarly high mortalities were not observed following dosing over Days 1–2 or 2–3 when a different formulation (10 g AI $litre^{-1}$) was used.

The changing feeding patterns within locust instars are likely to be important, especially for exposures of limited duration. Feeding is initially low after moulting, rises to a peak mid-instar and declines near moulting.[16,17] The low feeding rate at the start of the instar may allow a greater absorption of insecticide across the gut because throughput is low. This does not seem to be sufficient to retain the active ingredient until the most susceptible period, however.[7] Mid-instar feeding rates are higher, so greater exposure will occur, but high throughput means that the rate of uptake across the gut wall will be correspondingly reduced. Higher rates of chitin synthesis may provide more substrate for toxic action however and effects are increased. A late application falls inside the so called 'window of opportunity' of peak chitin deposition, and reduced feeding may allow time for more insecticide to cross the gut wall into the nymph's body and therefore give the higher mortality. If field application rates for BPUs against locusts are to be effectively estimated, they must be high enough to be toxic to early inter-moult nymphs after a single exposure.

The variable persistence and penetration rates mean that the application method may affect both the acute and chronic toxicity. Topical applications of diflubenzuron onto IV and V instar *S. gregaria*, for example, showed decreasing mortality the closer the nymphs were to the next moult,[18] suggesting that some developmental stages are more susceptible than others. With topical applications however, the same outcome would occur if the rate of chemical penetration was slow and toxic effects were delayed. This phenomenon could have important repercussions for the interpretation of published data where different exposure routes were used in toxicological tests. For example the findings of Mariy *et al.*[18] seem to contradict those of Yemane[16] who found that mortality of II and IV instar *S. gregaria* nymphs was greater when dosed orally late in the instar with diflubenzuron compared with early. These findings and those made in this study suggest the possibility that a 'window of opportunity' exists, during which nymphs are more susceptible to the toxin by ingestion than at other times.[7]

The effect that the timing and duration of exposure had on mortality class distribution further illustrates the potencies of the insecticides. Death tended to occur in moult after a single dose of hexaflumuron or teflubenzuron, whereas diflubenzuron-treated nymphs mostly died post-moult indicating that hexaflumuron and teflubenzuron are both more active than diflubenzuron. However, this difference was lost when doses were applied over more than one day, demonstrating that the inherently high toxicity of all three BPUs against *S. gregaria* nymphs is limited by their non-cumulative action. Relative rates of elimination and metabolism may explain the toxicological differences between the products. This apparent limitation is not an accurate reflection of field activity where insects are unlikely to encounter a single dose during the course of a single meal but will be exposed more continuously to the mass of treated vegetation. This will be of greatest benefit for diflubenzuron which has the greatest elimination and metabolism rates and may consequently prove as potent as hexaflumuron and teflubenzuron under field conditions.

The significantly increased duration of the II and III instars following many dose regimes supports similar findings made previously.[10,11] It is unlikely that prolonged instars will lead to nymphs consuming significantly more treated foliage because, in this work, most treated nymphs showed reduced feeding rates, especially during the III instar when many of the individuals that survived the first moult after exposure were very weak. In the harsh field environment one would therefore expect to find higher rates of mortality because weakened survivors would become more susceptible to prevailing conditions, predators and parasites.

5 CONCLUSIONS

The results show that increased duration of exposure leads to increased mortality. This method of exposure, to a single dose per day, is not representative of field conditions; it does however simulate, in a controlled way, the repeated exposures that are likely to occur in the field. The data indicate that much-reduced doses may be effective if exposure is prolonged, indicating scope for low rates of application in the field. The 'window of opportunity' will therefore be particularly important for the effectiveness of barrier spraying where nymphs may be exposed to the insecticide for variable periods. Thus, non-cumulative insecticides such as BPUs must be applied at sufficiently high doses to ensure that a lethal dose is consumed within one barrier. Barriers must also be sufficiently wide to ensure high enough rates of pesticide uptake. Blanket spraying over large areas, however, would require considerably lower dose rates than in barriers to achieve the same effect, because prolonged exposure increases mortality rates. In order to make accurate predictions for the application rates necessary to achieve control in barrier treatments, nymphs should be experimentally exposed to sprayed vegetation for varying periods of time to simulate their experience in crossing a barrier. More detailed observations need to be made and should include walking and feeding rates that could be incorporated into a model to predict chronic effects, such as reduced mobility, that may in turn raise acute effects by trapping nymphs within barriers.[5,6]

ACKNOWLEDGEMENTS

The authors would like to thank Kathy Ballard from Southampton University for technical assistance, Dow-Elanco, Shell Research Ltd, Solvay-Duphar BV for supplying the chemicals and the Science and Engineering Research Council for providing a grant for G. D. A. Coppen.

REFERENCES

1. van Daalen, J. J., Meltzer, J., Mulder, R. & Wellinga, K., A selective insecticide with a novel mode of action. *Naturwshftn*, **59** (1972) 312–13.
2. Onyeocha, F. A. & Fuzeau-Braesch, S., Comparative toxicity of some synthetic insecticides and a growth inhibitor in the Migratory locust, *Locusta migratoria*. *Comp. Biochem. Physiol.*, **100** (1991) 361–3.
3. Müller, P., *Die Untersuchungen im Sudan wurden Durchgeführt*. Deutschen Gesellschaft fur Technische Zusammenarbeit, Germany, 1988, 70 pp.
4. Sissoko, M., A novel experimental investigation of the persistence of Dimilin against grasshoppers. *MPhil. Thesis*, University of Southampton, UK, 1991, 160 pp.
5. Bouaichi, A., Coppen, G. D. A. & Jepson, P. C., Barrier spray treatment with diflubenzuron (ULV) against gregarious hopper bands of the Moroccan locust *Dociostaurus maroccanus* (Thunberg) (Orthoptera: Acrididae) in N.E. Morocco. *Crop Prot.*, **13** (1994) 60–72.
6. Cooper, J. F., Coppen, G. D. A., Dobson, H., Rakotonandrasana, A. & Scherer, R., Sprayed barriers of diflubenzuron (ULV) as a control technique against marching hopper bands of Migratory locust *Locusta migratoria capito* (Sauss.) (Orthoptera: Acrididae) in Southern Madagascar. *Crop Prot.*, **14** (1995) 137–44.
7. Ker, R. F., Investigation of locust cuticle using the insecticide diflubenzuron. *J. Insect Physiol.*, **23** (1977) 39–48.
8. Fisk, T. & Wright, D. J., Response of *Spodoptera exempta* (Walk.) larvae to simulated field spray applications of acylurea insect growth regulators with observations on cuticular uptake of acylureas. *Pestic. Sci.*, **35** (1992) 331–7.
9. Guyer, W. & Neuman, R., Activity and fate of chlorfluazuron and diflubenzuron in the larvae of *Spodoptera littoralis* and *Heliothis virescens*. *Pestic. Biochem. Physiol.*, **30** (1988) 166–77.
10. Coppen, G. D. A., The use of benzoylphenylureas as novel insecticides for the control of locusts and grasshoppers. *PhD thesis*, University of Southampton, UK, 1994, 190 pp.

11. Coppen, G. D. A. & Jepson, P. C., Comparative laboratory evaluation of the acute and chronic toxicology of diflubenzuron, hexflumuron and teflubenzuron against II instar Desert locust (*Schistocerca gregaria*) (Orthoptera: Acrididae). *Pestic. Sci.*, **46** (1996) 183–90.
12. Grosscurt, A. C., & Jongsma, B., Mode of action and insecticidal properties of diflubenzuron. In *Chitin and benzoylphenyl ureas*, ed. J. E. Wright & A. Retnakaran. Dr W. Junk Publ., Netherlands, 1987, pp. 75–99.
13. Fisk, T. & Wright, D. J., Comparative studies on acylurea insect growth regulators and neuroactive insecticides for the control of the armyworm *Spodoptera exempta* Walk. *Pestic. Sci.*, **35** (1992) 175–82.
14. Neuman, R. & Guyer, W., Biochemical and toxicological differences in the modes of action of the benzoylureas. *Pestic. Sci.*, **20** (1987) 147–56.
15. Gazit, Y., Ishaaya, I. & Perry, A. S., Detoxification and synergism of diflubenzuron and chlorfluazuron in the Red flour beetle *Tribolium castaneum*. *Pestic. Biochem. Physiol.*, **34** (1989) 103–10.
16. Yemane, A., A laboratory study of diflubenzuron to determine toxicity to Desert locust (*Schistocerca gregaria* Forskål) and to predict efficacy in the field. *MPhil. Thesis*, University of Southampton, UK, 1992, 150 pp.
17. Davey, P. M., Quantities of food eaten by the Desert locust, *Schistocerca gregaria* (Forsk.) in relation to growth. *Bull. Entomol. Res.*, **45** (1954) 539–51.
18. Mariy, F. M. A., Hussein, E. M. K., El Guindy, M. A. & Ibrahim, E. E. H., Studies on the biological effects of diflubenzuron on the Desert locust (*Schistocerca gregaria* Forskål). *Internat. Pest Control*, **23** (1981) 133–5.